PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 10 July 2000 (10.07.00)	
International application No. PCT/US99/24630	**Applicant's or agent's file reference** 17810-043
International filing date (day/month/year) 21 October 1999 (21.10.99)	**Priority date** (day/month/year) 26 October 1998 (26.10.98)
Applicant TAO, Weng et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

25 May 2000 (25.05.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Olivia RANAIVOJAONA

Telephone No.: (41-22) 338.83.38

MPlynn

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

ELRIFI, IVOR R.
Mintz,Levin, Cohn, Ferris, Glovsky
and Popeo, P.C.
One Financial Center
60 State Street
Boston, MA 02111
ETATS-UNIS D'AMERIQUE

FACSIMILE: 001-617-542-2241

PTO/PCT Rec'd 26 APR 2001

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	22.01.2001
Applicant's or agent's file reference ~~17810-043~~ 19141-543	IMPORTANT NOTIFICATION
International application No. PCT/US99/24630	International filing date *(day/month/year)* 21/10/1999
	Priority date *(day/month/year)* 26/10/1998
Applicant CYTOTHERAPEUTICS, INC. et al.	

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the International application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

3/16 per MF

RECEIVED
FEB 16 2001
MINTZ LEVIN, BOSTON
PATENT DOCKET DEPT.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Papiol Rovira, M Tel.+49 89 2399-7199

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
17810-043		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/24630	21/10/1999	26/10/1998
International Patent Classification (IPC) or national classification and IPC		
C12N15/13		
Applicant		
CYTOTHERAPEUTICS, INC. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
25/05/2000	22.01.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Trommsdorff, M Telephone No. +49 89 2399 7361

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US99/24630

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-31 as originally filed

Claims, No.:

1-30 as amended under Article 19

Drawings, sheets:

1/5-5/5 as originally filed

Sequence listing part of the description, pages:

1-16, filed with the demand

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US99/24630

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed.

   ☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

   Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:
   see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 16-28, 30.

because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US99/24630

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no International search report has been established for the said claims Nos. 16-28, 30.

2. A meaningful International preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-15, 29
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-15, 29
Industrial applicability (IA)	Yes:	Claims	1-15, 29
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

1. Cited documents

The following documents (D) are referred to in this communication; the numbering is the same as in the search report and will be adhered to in the rest of the procedure:

D1: YU X ET AL: 'The role of B7-CD28 co-stimulation in tumor rejection.' INTERNATIONAL IMMUNOLOGY, (1998 JUN) 10 (6) 791-7

D2: GAJEWSKI THOMAS F ET AL: 'Tumor rejection requires a CTLA4 ligand provided by the host or expressed on the tumor: Superiority of B7-1 over B7-2 for active tumor immunization.' JOURNAL OF IMMUNOLOGY 1996, vol. 156, no. 8, 1996, pages 2909-2917, ISSN: 0022-1767

D3: WO 98 02846 A (CYTOTHERAPEUTICS INC) 1 Feb 1996 (1996-02-01)

D4: STABILA P F ET AL: 'Cell surface expression of a human IgG Fc chimera activates macrophages through Fc receptors.' NATURE BIOTECHNOLOGY, (1998 DEC) 16 (13) 1357-60

2. Content of the application

The present application describes cells expressing recombinant polynucleotides encoding cell surface molecules that lead to the rejection of said cells by the host immune system. More specifically described are antibody-like chimeric polynucleotides encoding a truncated IgG gene (containing only the second and third domains of the heavy chain, i.e. CH2 and CH3) fused N-terminally to the transmembrane sequence of a type II membrane receptor such as, e.g. the transferrin receptor. Said fusion polypeptides (with a Fc portion expressed in a reverse orientation to the cell surface compared to the naturally occuring IgG) are claimed to activate phagocytes but not to fix complement. Furthermore, capsules for the delivery of said polypeptides to a patient are claimed.

3. Re Item II
Priority

The priority has been checked: the contents of the description of the priority document and of the application as filed appear to be the same.
The amendments filed (letter of 25.05.2000) with the International Bureau under

Article 19(1) do not appear to introduce subject-matter which extends beyond the content of the application as filed (Art.19(2) PCT).
Thus, the priority is valid and it is assumed that all the claims enjoy the claimed priority date.
Therefore, document D4 has not been considered to be part of the prior art as defined in the regulations (Rule 64(1)-(3) PCT).

4. Re Item III
Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The claims 16-28 and 30 (which correspond to originally filed claims 20-32 and 34) are directed to methods of treatment of the human or animal body and have not been searched (Rule 39.1(iv) PCT).
The applicant is advised that the EPO policy when acting as an International Preliminary Examination Authority is normally not to carry out a preliminary examination on matter which has not been searched (Rule 66.1(e) PCT). This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.
Thus, claims 16-28 and 30 were not examined.
For the further assessment of the present claims 16-28 and 30 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

5. Re Item V
Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Claim 1 and its dependent claims are directed to a cell transformed with a vector

encoding a fusion protein comprising an immunostimulatory cell surface polypeptide linked at its N-terminus to a second cell surface polypeptide containing a transmembrane region.

D1 shows how EL4 (T cell lymphoma) cells expressing full-length or truncated B7-1 or B7-2 molecules (members of the Ig supergene family) induce tumour rejection (p.791, right column; p.792, left column, 5th paragraph). Thus, said cells express immunostimulatory cell surface molecules, i.e. B7-1 or B7-2. However, said proteins are full-length or truncated proteins (lacking the cytoplasmic domain) and not fusion proteins.
Hence, claim 1 and its dependent claims 2-8 are novel over D1.

D2 describes cells transfected with a vector encoding a fusion protein comprising the extracellular domain of CTLA4 (receptor for B7-1) fused to a truncated IgG3 protein containing the hinge, the CH2 and the CH3 domains (p.2910, 2nd column, 2nd paragraph). Thus, said cells contain a vector encoding a fusion protein that is immunostimulatory. However, since the transmembrane region of CTLA4 is missing, said fusion protein is soluble and not expressed on the cell surface, i.e. not membrane bound.
Hence, the subject-matter of claim 1 is novel over D2 (Art. 33(2) PCT).
Claims 2-8 are dependent on claim1 and as such also meet the requirements of the PCT with respect to novelty.

Neither D1 nor D2, nor a combination of said documents suggest the design of a membrane bound immunostimulatory fusion protein as defined in claim 1. Thus, the subject-matter of claim 1 would not be derivable in an obvious manner from the prior art by the skilled person.
Hence, the subject-matter of claim 1 is considered as inventive over the prior art (Art. 33(3) PCT). The same applies mutatis mutandis to dependent claims 2-8.
Claim 9 is directed to a recombinant polynucleotide comprising a promoter operably linked with a polynucleotide coding for an immunostimulatory cell surface polypeptide, wherein said polypeptide activates phagocytes but does not fix complement.
As mentioned previously, the fusion protein described in D2 lacks the CH1 region but contains the CH2 and CH3 regions. Thus, said fusion protein contains the

same IgG domains as the fusion protein described in the present application (p.22-p.30, example 1 of the application, as filed). It could thereby implicitly have the same biological activity, i.e. to activate phagocytes and not to fix complement. However, the fusion protein of D2 differs from the cell surface protein claimed in claim 9 in that it lacks a transmembrane domain and is thus soluble.
Thus, claim 9 is novel.
The design of a polynucleotide encoding a membrane bound immunostimulatory protein is not obvious to the skilled person from the sequences disclosed in D1 or D2. Thus, claim 9 is also inventive over the prior art.

Claim 10 is directed to an immunostimulatory cell surface protein comprising a cell surface Fc expressed in reverse orientation to the cell surface.
None of the cited prior art documents discloses or anticipates a polypeptide with said technical features.
Thus, the subject-matter of claim 10 is novel and inventive.
Claims 11 and 12 are dependent on claim10 and as such also meet the requirements of the PCT with respect to novelty and inventive step.

Claims 13-15 are method claims that comprise the step of contacting a phagocyte or a test agent with " a transformed cell expressing the immunostimulatory cell surface polypeptide in reverse orientation to the cell surface".
Since a cell with said technical features has not been disclosed in the cited prior art, said claims are novel.
Moreover, the concept of expressing an immunostimmulatory cell surface polypeptide in a reverse orientation is not anticipated by any prior art document and is therefore inventive. Thus, the use of such a polypeptide in a method renders said method also inventive.
Hence, claims 13-15 are inventive.

Claim 29 is directed to a composition comprising a core containing a transformed cell expressing an immunostimulatory polypeptide capable of stimulating an immune response against the cell in a host and a jacket surrounding said core comprising a permselective membrane.
Such a composition has not been disclosed in any of the cited prior art documents previously. Hence, the subject-matter of claim 29 is novel.

D3 describes the encapsulation of cells in bioartificial organs to avoid their immunological rejection by the host organ (p.10, l.13-30). Said cells can deliver biologically active molecules to the host through the permselective membrane, the pore size being selected to exclude the entry of host immune response factors (p.41, l.30-p.42, l.13). The biologically active molecule "may be expressed on the cell surface" or "may be released or secreted...such as, e.g., a cytokine " (p.10, l.31-p.11, l.11), cytokines being known to be involved in the regulation of the immune response.
Hence, D3 describes cells encapsulated in a jacket comprising a permselective membrane and expressing biologically active molecules that can be membrane bound or affect the immune response of the host.
Thus, the only difference between claim 29 and D3 appears to reside in the nature of the "biologically active molecule" expressed by the encapsulated cells, i.e. the claimed molecule being able to stimulate an immune response against the cell.
Hence, the problem to be solved by the skilled person is to express such an immunostimulatory molecule in the encapsulated cells.
However, most of the xenogenic molecules expressed by implanted cells in a host will lead to an immunological rejection by the host, since this is precisely the reason why said cells are encapsulated. Thus, the skilled person could choose a molecule among many of the known membrane bound molecules to express it in the encapsulated cells and would very likely see an immune response in the host.
Hence, no inventive step can be seen for the subject-matter of claim 29.

6. Re Item VIII
Certain observations on the international application

6.1. The expression "a transferrin receptor hinge region" of claims 8 and 12 is vague in that it does not clearly define the region meant. It would be suggested to specify said expression by, e.g. adding the references of the primers used to amplify said region (Art. 6 PCT).

6.2. It would be suggested to clarify the rather vague expression "expressed in reverse orientation to the cell surface" of claims 10, 13-15, by adding "compared to a naturally occuring Fc".

PATENT COOPERATION TREA

PCT Rec'd 26 APR 2001

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

To:
Mintz,Levin, Cohn, Ferris, Glovsky and Popeo, P.C.
Attn. ELRIFI, IVOR R.
One Financial Center
60 State Street
Boston, MA 02111
UNITED STATES OF AMERICA

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NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

Applicant's or agent's file reference ~~17810-043~~ 19141-5A3 WO	FOR FURTHER ACTION See paragraphs 1 and 4 below
International application No. PCT/US 99/24630	International filing date (day/month/year) 21/10/1999
Applicant CYTOTHERAPEUTICS, INC. et al.	

Date of mailing (day/month/year) 06/04/2000

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. ☐ The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. ☐ **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Nina Vercio

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international pbulication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international appplication is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREA\ .

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
17810-043		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 99/24630	21/10/1999	26/10/1998
Applicant		
CYTOTHERAPEUTICS, INC. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [X] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [X] furnished subsequently to this Authority in computer readble form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
[redacted]/US 99/24630

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 20-32, 34
because they relate to subject matter not required to be searched by this Authority, namely:
Rule 39.1 (iv) PCT - Method for treatment of the human or animal body by therapy

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/24630

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/13 C12N5/10 C12N15/62 G01N33/53 A61K45/00
A61K9/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YU X ET AL: "The role of B7-CD28 co-stimulation in tumor rejection." INTERNATIONAL IMMUNOLOGY, (1998 JUN) 10 (6) 791-7. , XP000891283 abstract figures 3,4 page 796, right-hand column, paragraph 2 --- -/--	1-4

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 March 2000	06/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Covone, M

1

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GAJEWSKI THOMAS F ET AL: "Tumor rejection requires a CTLA4 ligand provided by the host or expressed on the tumor: Superiority of B7-1 over B7-2 for active tumor immunization." JOURNAL OF IMMUNOLOGY 1996, vol. 156, no. 8, 1996, pages 2909-2917, XP002133519 ISSN: 0022-1767 page 2909, left-hand column, line 12 -right-hand column, line 17 page 2913, left-hand column, paragraph 3 -right-hand column, paragraph 3 table 1	1-4
A	WO 96 02646 A (CYTOTHERAPEUTICS INC) 1 February 1996 (1996-02-01) page 6, line 18 -page 7, line 22 page 15, line 13 -page 24, line 6 claims 1,2,31	1-34
P,X	STABILA P F ET AL: "Cell surface expression of a human IgG Fc chimera activates macrophages through Fc receptors." NATURE BIOTECHNOLOGY, (1998 DEC) 16 (13) 1357-60. , XP002133520 the whole document	1-34

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 99/24630

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9602646 A	01-02-1996	US 5935849 A	10-08-1999
		US 5843431 A	01-12-1998
		AU 698624 B	05-11-1998
		AU 3142295 A	16-02-1996
		AU 708186 B	29-07-1999
		AU 8424098 A	05-11-1998
		BR 9508312 A	01-06-1999
		CA 2195446 A	01-02-1996
		CN 1152938 A	25-06-1997
		CZ 9700163 A	18-03-1998
		EP 0771350 A	07-05-1997
		FI 970217 A	17-01-1997
		HU 77875 A	28-09-1998
		JP 10506266 T	23-06-1998
		NO 970156 A	20-03-1997
		NZ 290629 A	28-10-1998
		PL 318288 A	09-06-1997
		SK 7797 A	06-08-1997
		TR 960076 A	21-06-1998
		US 5840576 A	24-11-1998
		US 5853717 A	29-12-1998
		US 5833979 A	10-11-1998
		US 5776747 A	07-07-1998
		US 5858747 A	12-01-1999
		US 5795790 A	18-08-1998
		ZA 9505721 A	21-02-1996

PATENT COOPERATION TREATY

RECD 24 JAN 2001

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

15

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
17810-043		

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/24630	21/10/1999	26/10/1998

International Patent Classification (IPC) or national classification and IPC
C12N15/13

Applicant
CYTOTHERAPEUTICS, INC. et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
25/05/2000	22.01.2001

Name and mailing address of the international preliminary examining authority:

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Authorized officer

Trommsdorff, M

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US99/24630

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-31 as originally filed

Claims, No.:

1-30 as amended under Article 19

Drawings, sheets:

1/5-5/5 as originally filed

Sequence listing part of the description, pages:

1-16, filed with the demand

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed.

   ☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

   Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:
   see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 16-28, 30.

because:

- ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

- ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 16-28, 30.

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-15, 29
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-15, 29
Industrial applicability (IA)	Yes:	Claims	1-15, 29
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

1. Cited documents

The following documents (D) are referred to in this communication; the numbering is the same as in the search report and will be adhered to in the rest of the procedure:

D1: YU X ET AL: 'The role of B7-CD28 co-stimulation in tumor rejection.' INTERNATIONAL IMMUNOLOGY, (1998 JUN) 10 (6) 791-7

D2: GAJEWSKI THOMAS F ET AL: 'Tumor rejection requires a CTLA4 ligand provided by the host or expressed on the tumor: Superiority of B7-1 over B7-2 for active tumor immunization.' JOURNAL OF IMMUNOLOGY 1996, vol. 156, no. 8, 1996, pages 2909-2917, ISSN: 0022-1767

D3: WO 96 02646 A (CYTOTHERAPEUTICS INC) 1 Feb 1996 (1996-02-01)

D4: STABILA P F ET AL: 'Cell surface expression of a human IgG Fc chimera activates macrophages through Fc receptors.' NATURE BIOTECHNOLOGY, (1998 DEC) 16 (13) 1357-60

2. Content of the application

The present application describes cells expressing recombinant polynucleotides encoding cell surface molecules that lead to the rejection of said cells by the host immune system. More specifically described are antibody-like chimeric polynucleotides encoding a truncated IgG gene (containing only the second and third domains of the heavy chain, i.e. CH2 and CH3) fused N-terminally to the transmembrane sequence of a type II membrane receptor such as, e.g. the transferrin receptor. Said fusion polypeptides (with a Fc portion expressed in a reverse orientation to the cell surface compared to the naturally occuring IgG) are claimed to activate phagocytes but not to fix complement. Furthermore, capsules for the delivery of said polypeptides to a patient are claimed.

3. Re Item II
Priority

The priority has been checked: the contents of the description of the priority document and of the application as filed appear to be the same.
The amendments filed (letter of 25.05.2000) with the International Bureau under

Article 19(1) do not appear to introduce subject-matter which extends beyond the content of the application as filed (Art.19(2) PCT).
Thus, the priority is valid and it is assumed that all the claims enjoy the claimed priority date.
Therefore, document D4 has not been considered to be part of the prior art as defined in the regulations (Rule 64(1)-(3) PCT).

4. Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The claims 16-28 and 30 (which correspond to originally filed claims 20-32 and 34) are directed to methods of treatment of the human or animal body and have not been searched (Rule 39.1(iv) PCT).
The applicant is advised that the EPO policy when acting as an International Preliminary Examination Authority is normally not to carry out a preliminary examination on matter which has not been searched (Rule 66.1(e) PCT). This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.
Thus, claims 16-28 and 30 were not examined.
For the further assessment of the present claims 16-28 and 30 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

5. Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Claim 1 and its dependent claims are directed to a cell transformed with a vector

encoding a fusion protein comprising an immunostimulatory cell surface polypeptide linked at its N-terminus to a second cell surface polypeptide containing a transmembrane region.

D1 shows how EL4 (T cell lymphoma) cells expressing full-length or truncated B7-1 or B7-2 molecules (members of the Ig supergene family) induce tumour rejection (p.791, right column; p.792, left column, 5th paragraph). Thus, said cells express immunostimulatory cell surface molecules, i.e. B7-1 or B7-2. However, said proteins are full-length or truncated proteins (lacking the cytoplasmic domain) and not fusion proteins.
Hence, claim 1 and its dependent claims 2-8 are novel over D1.

D2 describes cells transfected with a vector encoding a fusion protein comprising the extracellular domain of CTLA4 (receptor for B7-1) fused to a truncated IgG3 protein containing the hinge, the CH2 and the CH3 domains (p.2910, 2nd column, 2nd paragraph). Thus, said cells contain a vector encoding a fusion protein that is immunostimulatory. However, since the transmembrane region of CTLA4 is missing, said fusion protein is soluble and not expressed on the cell surface, i.e. not membrane bound.
Hence, the subject-matter of claim 1 is novel over D2 (Art. 33(2) PCT).
Claims 2-8 are dependent on claim1 and as such also meet the requirements of the PCT with respect to novelty.

Neither D1 nor D2, nor a combination of said documents suggest the design of a membrane bound immunostimulatory fusion protein as defined in claim 1. Thus, the subject-matter of claim 1 would not be derivable in an obvious manner from the prior art by the skilled person.
Hence, the subject-matter of claim 1 is considered as inventive over the prior art (Art. 33(3) PCT). The same applies mutatis mutandis to dependent claims 2-8.
Claim 9 is directed to a recombinant polynucleotide comprising a promoter operably linked with a polynucleotide coding for an immunostimulatory cell surface polypeptide, wherein said polypeptide activates phagocytes but does not fix complement.
As mentioned previously, the fusion protein described in D2 lacks the CH1 region but contains the CH2 and CH3 regions. Thus, said fusion protein contains the

same IgG domains as the fusion protein described in the present application (p.22-p.30, example 1 of the application, as filed). It could thereby implicitly have the same biological activity, i.e. to activate phagocytes and not to fix complement. However, the fusion protein of D2 differs from the cell surface protein claimed in claim 9 in that it lacks a transmembrane domain and is thus soluble.
Thus, claim 9 is novel.
The design of a polynucleotide encoding a membrane bound immunostimulatory protein is not obvious to the skilled person from the sequences disclosed in D1 or D2. Thus, claim 9 is also inventive over the prior art.

Claim 10 is directed to an immunostimulatory cell surface protein comprising a cell surface Fc expressed in reverse orientation to the cell surface.
None of the cited prior art documents discloses or anticipates a polypeptide with said technical features.
Thus, the subject-matter of claim 10 is novel and inventive.
Claims 11 and 12 are dependent on claim10 and as such also meet the requirements of the PCT with respect to novelty and inventive step.

Claims 13-15 are method claims that comprise the step of contacting a phagocyte or a test agent with " a transformed cell expressing the immunostimulatory cell surface polypeptide in reverse orientation to the cell surface".
Since a cell with said technical features has not been disclosed in the cited prior art, said claims are novel.
Moreover, the concept of expressing an immunostimmulatory cell surface polypeptide in a reverse orientation is not anticipated by any prior art document and is therefore inventive. Thus, the use of such a polypeptide in a method renders said method also inventive.
Hence, claims 13-15 are inventive.

Claim 29 is directed to a composition comprising a core containing a transformed cell expressing an immunostimulatory polypeptide capable of stimulating an immune response against the cell in a host and a jacket surrounding said core comprising a permselective membrane.
Such a composition has not been disclosed in any of the cited prior art documents previously. Hence, the subject-matter of claim 29 is novel.

D3 describes the encapsulation of cells in bioartificial organs to avoid their immunological rejection by the host organ (p.10, l.13-30). Said cells can deliver biologically active molecules to the host through the permselective membrane, the pore size being selected to exclude the entry of host immune response factors (p.41, l.30-p.42, l.13). The biologically active molecule "may be expressed on the cell surface" or "may be released or secreted...such as, e.g., a cytokine " (p.10, l.31-p.11, l.11), cytokines being known to be involved in the regulation of the immune response.
Hence, D3 describes cells encapsulated in a jacket comprising a permselective membrane and expressing biologically active molecules that can be membrane bound or affect the immune response of the host.
Thus, the only difference between claim 29 and D3 appears to reside in the nature of the "biologically active molecule" expressed by the encapsulated cells, i.e. the claimed molecule being able to stimulate an immune response against the cell.
Hence, the problem to be solved by the skilled person is to express such an immunostimulatory molecule in the encapsulated cells.
However, most of the xenogenic molecules expressed by implanted cells in a host will lead to an immunological rejection by the host, since this is precisely the reason why said cells are encapsulated. Thus, the skilled person could choose a molecule among many of the known membrane bound molecules to express it in the encapsulated cells and would very likely see an immune response in the host.
Hence, no inventive step can be seen for the subject-matter of claim 29.

6. Re Item VIII
Certain observations on the international application

6.1. The expression "a transferrin receptor hinge region" of claims 8 and 12 is vague in that it does not clearly define the region meant. It would be suggested to specify said expression by, e.g. adding the references of the primers used to amplify said region (Art. 6 PCT).

6.2. It would be suggested to clarify the rather vague expression "expressed in reverse orientation to the cell surface" of claims 10, 13-15, by adding "compared to a naturally occuring Fc".

CLAIMS

1. A cell transformed *in vitro* comprising a vector, the vector comprising a promoter operatively linked to a polynucleotide sequence encoding a fusion protein comprising immunostimulatory cell surface polypeptide linked at the amino terminus to second cell surface polypeptide, wherein the second cell surface polypeptide comprises a transmembrane region, wherein upon expression, the fusion protein is expressed on the cell surface.

2. The transformed cell of claim 1, wherein the immunostimulatory cell surface polypeptide
   (a) activates phagocytes; but
   (b) does not fix complement.

3. The transformed cell of claim 1, wherein the cell is human.

4. The transformed cell of claim 1, wherein the cell is rodent.

5. The transformed cell of claim 4, wherein the rodent cell is a hamster cell.

6. The transformed cell of claim 1, wherein the immunostimulatory cell surface polypeptide is a region of IgG.

7. The transformed cell of claim 6, wherein the region of IgG is Fc.

8. The transformed cell of claim 1, wherein the second cell surface polypeptide is a transferrin receptor hinge region.

9. A recombinant polynucleotide comprising a promoter operably linked with a polynucleotide coding for an immunostimulatory cell surface polypeptide, wherein the immunostimulatory cell surface polypeptide:
   (a) activates phagocytes; but
   (b) does not fix complement.

10. An immunostimulatory cell surface polypeptide, comprising a cell surface Fc expressed in reverse orientation to the cell surface.

11. The immunostimulatory cell surface polypeptide of claim 10, wherein the polypeptide further comprises a second cell surface polypeptide.

12. The immunostimulatory cell surface polypeptide of claim 11, wherein the second cell surface polypeptide is a transferrin receptor hinge region.

13. A screening method for testing phagocytes for response to an immunostimulatory cell surface polypeptide, comprising:

(a) contacting a phagocyte *in vitro* with a transformed cell expressing the immunostimulatory cell surface polypeptide in reverse orientation to the cell surface; and

(b) comparing the phagocytic activity of the phagocyte as compared with control phagocyte, wherein increased phagocytic activity indicates that the phagocyte responds to the immunostimulatory cell surface polypeptide in reverse orientation to the cell surface.

14. A method for identifying an agent that modulates phagocyte response to an immunostimulatory cell surface polypeptide, comprising:

(a) contacting a phagocyte *in vitro* with a transformed cell expressing the immunostimulatory cell surface polypeptide in reverse orientation to the cell surface;

(b) contacting a phagocyte *in vitro* with a test agent and the transformed cell expressing the immunostimulatory cell surface polypeptide in reverse orientation to the cell surface; and

(c) comparing the phagocytic activity of the phagocyte in the absence of the test agent with the phagocytic activity of the phagocyte in the presence of the test agent, wherein a change in the phagocytic activity indicates that the test agent modulates phagocyte response to the immunostimulatory cell surface polypeptide in reverse orientation to the cell surface.

15. A method for identifying an agent that preferentially binds to a cell surface orientation of an immunostimulatory cell surface polypeptide, comprising:

(a) contacting a test agent *in vitro* with a transformed cell expressing the immunostimulatory cell surface polypeptide in a type I cell surface protein;

(b) contacting the test agent *in vitro* with a transformed cell expressing the immunostimulatory cell surface polypeptide in reverse orientation; and

(c) comparing the effect of contacting the test agent with a transformed cell expressing the immunostimulatory cell surface polypeptide in a type I cell surface protein with the effect of contacting the test agent with a transformed cell expressing the immunostimulatory cell surface polypeptide in reverse orientation, wherein a change in effect indicates that the test agent preferentially binds to the cell surface orientation of the immunostimulatory cell surface polypeptide.

16. A method for stimulating phagocyte activity, comprising:

administering to the host a transformed cell containing a recombinant polynucleotide comprising a promoter operably linked with a polynucleotide coding for an immunostimulatory cell surface polypeptide, wherein the immunostimulatory cell surface polypeptide

(a) activates phagocytes; but

(b) does not fix complement,

wherein the administration of the transformed cell stimulates phagocyte activity by the phagocyte.

17. The method of claim 16, wherein the phagocyte is a macrophage.

18. The method of claim 16, wherein the phagocyte is a macrophagic tumor cell.

19. The method of claim 16, wherein the transformed cell comprises a therapeutic compound.

20. The method of claim 16, wherein the transformed cell is administered to the host central nervous system.

21. A method for modulating a host immune response, comprising:

administering to the host a transformed cell containing a recombinant polynucleotide comprising a promoter operably linked with a polynucleotide coding for an immunostimulatory cell surface polypeptide,

wherein the administration stimulates a host immune response to the transformed cell.

22. The method of claim 21, wherein the cell expresses, on the cell surface, a second antigen, such that the host produces an immune response against the second antigen.

23. The method of claim 22, wherein the cell expresses the second antigen from a recombinant polynucleotide.

24. A method for ablating target cells from a host, comprising:

(a) introducing into the target cell a recombinant polynucleotide comprising a promoter operably linked with a polynucleotide coding for an immunostimulatory cell surface polypeptide, wherein the immunostimulatory cell surface polypeptide

(i) activates phagocytes; but

(ii) does not fix complement;

(b) expressing the immunostimulatory cell surface polypeptide in the target cell, wherein the target cell is in the host;

wherein the expression of the immunostimulatory cell surface polypeptide by the target cell in a host induces a host phagocyte-mediated ablation of the target cell.

25. The method of claim 24, wherein the cells are tumor cells.

26. The method of claim 24, wherein the expression of the immunostimulatory cell surface polypeptide is constitutive.

27. The method of claim 24, wherein the expression of the immunostimulatory cell surface polypeptide is induced.

28. A method for treating an autoimmune disorder in a host, comprising:

(a) administering, to a host with an autoimmune disorder, transformed cells containing a recombinant polynucleotide comprising a promoter operably linked with a polynucleotide coding for an immunostimulatory cell surface polypeptide;

(b) expressing a therapeutically effective amount of immunostimulatory cell surface polypeptide from the recombinant polypeptide;

(c) contacting phagocytes with the immunostimulatory cell surface polypeptide, wherein the contacted phagocytes modulate the autoreactive T-cells to reduce T-cell autoreactivity in the host.

29. A composition comprising:

(a) a core comprising a transformed cell containing a recombinant polynucleotide comprising a promoter operably linked with a polynucleotide coding for an immunostimulatory cell surface polypeptide that is capable of stimulating an immune response against the cell in a host; and

(b) a jacket surrounding the core, the jacket comprising a permselective membrane.

30. A method for delivering a biologically active molecule to a patient, comprising:

implanting the patient with a capsule, the capsule having:

(a) a core comprising a transformed cell containing a recombinant polynucleotide comprising a promoter operably linked with a polynucleotide coding for an immunostimulatory cell surface polypeptide that is capable of stimulating an immune response against the cell in a host; and

(b) a jacket surrounding the core, the jacket comprising a permselective membrane,

wherein the transformed cell secretes a biologically active molecule from the capsule.

REPLACED BY CLAIMS
ART 34 AMDT 26 APR 2001

We claim:

1. A transformed cell containing a recombinant polynucleotide comprising a promoter operably linked with a polynucleotide coding for an immunostimulatory cell surface polypeptide, wherein expression of the immunostimulatory cell surface polypeptide induces the removal of the transformed cell from a host in which the transformed cell is located.

2. The transformed cell of claim 1, wherein the host is human.

3. The transformed cell of claim 1, wherein the host is rodent.

4. The transformed cell of claim 3, wherein the rodent is mouse.

5. A transformed cell containing a recombinant polynucleotide comprising a promoter operably linked with a polynucleotide coding for an immunostimulatory cell surface polypeptide, wherein the immunostimulatory cell surface polypeptide
   (a) activates phagocytes; but
   (b) does not fix complement.

6. The transformed cell of claim 5, wherein the cell is human.

7. The transformed cell of claim 5, wherein the cell is rodent.

8. The transformed cell of claim 7, wherein the rodent is hamster.

9. The transformed cell of claim 5, wherein the immunostimulatory cell surface polypeptide is a region of IgG.

10. The transformed cell of claim 9, wherein the region of IgG is Fc.

11. The transformed cell of claim 5, wherein the cell surface polypeptide further comprises a second cell surface polypeptide.

12. The transformed cell of claim 11, wherein the second cell surface polypeptide is a transferrin receptor hinge region.

13. A recombinant polynucleotide comprising a promoter operably linked with a polynucleotide coding for an immunostimulatory cell surface polypeptide, wherein the immunostimulatory cell surface polypeptide:
    (a) activates phagocytes; but
    (b) does not fix complement.

14. An immunostimulatory cell surface polypeptide, comprising a cell surface Fc expressed in reverse orientation to the cell surface.

15. The immunostimulatory cell surface polypeptide of claim 14, wherein the polypeptide further comprises a second cell surface polypeptide.

16. The immunostimulatory cell surface polypeptide of claim 15, wherein the second cell surface polypeptide is a transferrin receptor hinge region.

17. A screening method for testing phagocytes for response to an immunostimulatory cell surface polypeptide, comprising:
    (a) contacting a phagocyte *in vitro* with a transformed cell expressing the immunostimulatory cell surface polypeptide in reverse orientation to the cell surface; and
    (b) comparing the phagocytic activity of the phagocyte as compared with control phagocyte, wherein increased phagocytic activity indicates that the phagocyte responds to the immunostimulatory cell surface polypeptide in reverse orientation to the cell surface.

18. A method for identifying an agent that modulates phagocyte response to an immunostimulatory cell surface polypeptide, comprising:

(a) contacting a phagocyte *in vitro* with a transformed cell expressing the immunostimulatory cell surface polypeptide in reverse orientation to the cell surface;

(b) contacting a phagocyte *in vitro* with a test agent and the transformed cell expressing the immunostimulatory cell surface polypeptide in reverse orientation to the cell surface; and

(c) comparing the phagocytic activity of the phagocyte in the absence of the test agent with the phagocytic activity of the phagocyte in the presence of the test agent, wherein a change in the phagocytic activity indicates that the test agent modulates phagocyte response to the immunostimulatory cell surface polypeptide in reverse orientation to the cell surface.

19. A method for identifying an agent that preferentially binds to a cell surface orientation of an immunostimulatory cell surface polypeptide, comprising:

(a) contacting a test agent *in vitro* with a transformed cell expressing the immunostimulatory cell surface polypeptide in a type I cell surface protein;

(b) contacting the test agent *in vitro* with a transformed cell expressing the immunostimulatory cell surface polypeptide in reverse orientation; and

(c) comparing the effect of contacting the test agent with a transformed cell expressing the immunostimulatory cell surface polypeptide in a type I cell surface protein with the effect of contacting the test agent with a transformed cell expressing the immunostimulatory cell surface polypeptide in reverse orientation, wherein a change in effect indicates that the test agent preferentially binds to the cell surface orientation of the immunostimulatory cell surface polypeptide.

20. A method for stimulating phagocyte activity, comprising:

administering to the host a transformed cell containing a recombinant polynucleotide comprising a promoter operably linked with a polynucleotide coding for an immunostimulatory cell surface polypeptide, wherein the immunostimulatory cell surface polypeptide

(a) activates phagocytes; but

(b) does not fix complement,

wherein the administration of the transformed cell stimulates phagocyte activity by the phagocyte.

21. The method of claim 20, wherein the phagocyte is a macrophage.

22. The method of claim 20, wherein the phagocyte is a macrophagic tumor cell.

23. The method of claim 20, wherein the transformed cell comprises a therapeutic compound.

24. The method of claim 20, wherein the transformed cell is administered to the host central nervous system.

25. A method for modulating a host immune response, comprising:

administering to the host a transformed cell containing a recombinant polynucleotide comprising a promoter operably linked with a polynucleotide coding for an immunostimulatory cell surface polypeptide,

wherein the administration stimulates a host immune response to the transformed cell.

26. The method of claim 25, wherein the cell expresses, on the cell surface, a second antigen, such that the host produces an immune response against the second antigen.

27. The method of claim 23, wherein the cell expresses the second antigen from a recombinant polynucleotide.

28. A method for ablating target cells from a host, comprising:

(a) introducing into the target cell a recombinant polynucleotide comprising a promoter operably linked with a polynucleotide coding for an immunostimulatory cell surface polypeptide, wherein the immunostimulatory cell surface polypeptide

(i) activates phagocytes; but

(ii) does not fix complement;

(b) expressing the immunostimulatory cell surface polypeptide in the target cell, wherein the target cell is in the host;

wherein the expression of the immunostimulatory cell surface polypeptide by the target cell in a host induces a host phagocyte-mediated ablation of the target cell.

29. The method of claim 28, wherein the cells are tumor cells.

30. The method of claim 28, wherein the expression of the immunostimulatory cell surface polypeptide is constitutive.

31. The method of claim 28, wherein the expression of the immunostimulatory cell surface polypeptide is induced.

32. A method for treating an autoimmune disorder in a host, comprising:

(a) administering, to a host with an autoimmune disorder, transformed cells containing a recombinant polynucleotide comprising a promoter operably linked with a polynucleotide coding for an immunostimulatory cell surface polypeptide;

(b) expressing a therapeutically effective amount of immunostimulatory cell surface polypeptide from the recombinant polypeptide;

(c) contacting phagocytes with the immunostimulatory cell surface polypeptide, wherein the contacted phagocytes modulate the autoreactive T-cells to reduce T-cell autoreactivity in the host.

33. A composition comprising:

(a) a core comprising a transformed cell containing a recombinant polynucleotide comprising a promoter operably linked with a polynucleotide coding for an

immunostimulatory cell surface polypeptide that is capable of stimulating an immune response against the cell in a host; and

(b) a jacket surrounding the core, the jacket comprising a permselective membrane.

34. A method for delivering a biologically active molecule to a patient, comprising:

implanting the patient with a capsule, the capsule having:

(a) a core comprising a transformed cell containing a recombinant polynucleotide comprising a promoter operably linked with a polynucleotide coding for an immunostimulatory cell surface polypeptide that is capable of stimulating an immune response against the cell in a host; and

(b) a jacket surrounding the core, the jacket comprising a permselective membrane,

wherein the transformed cell secretes a biologically active molecule from the capsule.

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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A1

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(74) Agent: ELRIFI, Ivor, R.; Mintz, Levin, Cohn, Ferris, Glovsky and Popeo, P. C., One Financial Center, Boston, MA 02111 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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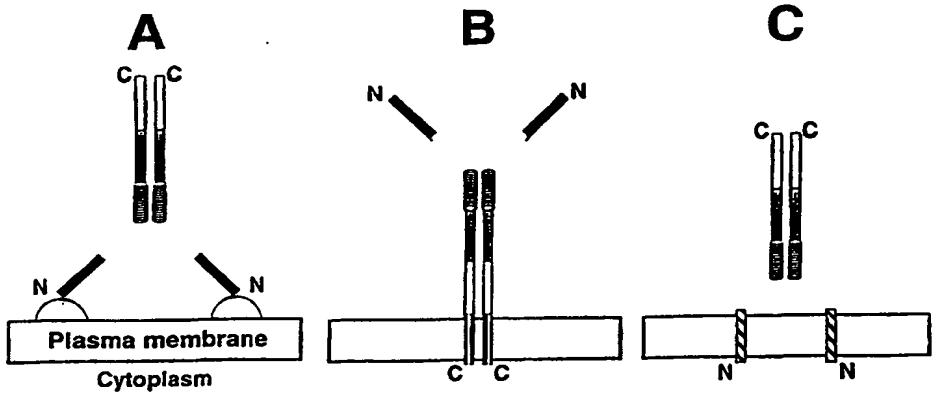
With amended claims and statement.

Date of publication of the amended claims and statement:
13 July 2000 (13.07.00)

(54) Title: CELL SURFACE MOLECULE–INDUCED MACROPHAGE ACTIVATION



(57) Abstract

This invention provides cells containing recombinant polynucleotides coding for cell surface molecules that, when expressed in the cell, result in rejection of the cell by the host immune system. The invention also provides methods of using such cells, and capsules for delivery of biologically active molecules to a patient.

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AMENDED CLAIMS

[received by the International Bureau on 25 May 2000 (25.05.00);
original claims 1-34 replaced by amended claims 1-30 (5 pages)]

1. A cell transformed *in vitro* comprising a vector, the vector comprising a promoter operatively linked to a polynucleotide sequence encoding a fusion protein comprising immunostimulatory cell surface polypeptide linked at the amino terminus to second cell surface polypeptide, wherein the second cell surface polypeptide comprises a transmembrane region, wherein upon expression, the fusion protein is expressed on the cell surface.

2. The transformed cell of claim 1, wherein the immunostimulatory cell surface polypeptide
   (a) activates phagocytes; but
   (b) does not fix complement.

3. The transformed cell of claim 1, wherein the cell is human.

4. The transformed cell of claim 1, wherein the cell is rodent.

5. The transformed cell of claim 4, wherein the rodent cell is a hamster cell.

6. The transformed cell of claim 1, wherein the immunostimulatory cell surface polypeptide is a region of IgG.

7. The transformed cell of claim 6, wherein the region of IgG is Fc.

8. The transformed cell of claim 1, wherein the second cell surface polypeptide is a transferrin receptor hinge region.

9. A recombinant polynucleotide comprising a promoter operably linked with a polynucleotide coding for an immunostimulatory cell surface polypeptide, wherein the immunostimulatory cell surface polypeptide:
   (a) activates phagocytes; but
   (b) does not fix complement.

10. An immunostimulatory cell surface polypeptide, comprising a cell surface Fc expressed in reverse orientation to the cell surface.

11. The immunostimulatory cell surface polypeptide of claim 10, wherein the polypeptide further comprises a second cell surface polypeptide.

12. The immunostimulatory cell surface polypeptide of claim 11, wherein the second cell surface polypeptide is a transferrin receptor hinge region.

13. A screening method for testing phagocytes for response to an immunostimulatory cell surface polypeptide, comprising:

(a) contacting a phagocyte *in vitro* with a transformed cell expressing the immunostimulatory cell surface polypeptide in reverse orientation to the cell surface; and

(b) comparing the phagocytic activity of the phagocyte as compared with control phagocyte, wherein increased phagocytic activity indicates that the phagocyte responds to the immunostimulatory cell surface polypeptide in reverse orientation to the cell surface.

14. A method for identifying an agent that modulates phagocyte response to an immunostimulatory cell surface polypeptide, comprising:

(a) contacting a phagocyte *in vitro* with a transformed cell expressing the immunostimulatory cell surface polypeptide in reverse orientation to the cell surface;

(b) contacting a phagocyte *in vitro* with a test agent and the transformed cell expressing the immunostimulatory cell surface polypeptide in reverse orientation to the cell surface; and

(c) comparing the phagocytic activity of the phagocyte in the absence of the test agent with the phagocytic activity of the phagocyte in the presence of the test agent, wherein a change in the phagocytic activity indicates that the test agent modulates phagocyte response to the immunostimulatory cell surface polypeptide in reverse orientation to the cell surface.

15. A method for identifying an agent that preferentially binds to a cell surface orientation of an immunostimulatory cell surface polypeptide, comprising:

(a) contacting a test agent *in vitro* with a transformed cell expressing the immunostimulatory cell surface polypeptide in a type I cell surface protein;

(b) contacting the test agent *in vitro* with a transformed cell expressing the immunostimulatory cell surface polypeptide in reverse orientation; and

(c) comparing the effect of contacting the test agent with a transformed cell expressing the immunostimulatory cell surface polypeptide in a type I cell surface protein with the effect of contacting the test agent with a transformed cell expressing the immunostimulatory cell surface polypeptide in reverse orientation, wherein a change in effect indicates that the test agent preferentially binds to the cell surface orientation of the immunostimulatory cell surface polypeptide.

16. A method for stimulating phagocyte activity, comprising:

administering to the host a transformed cell containing a recombinant polynucleotide comprising a promoter operably linked with a polynucleotide coding for an immunostimulatory cell surface polypeptide, wherein the immunostimulatory cell surface polypeptide

(a) activates phagocytes; but

(b) does not fix complement,

wherein the administration of the transformed cell stimulates phagocyte activity by the phagocyte.

17. The method of claim 16, wherein the phagocyte is a macrophage.

18. The method of claim 16, wherein the phagocyte is a macrophagic tumor cell.

19. The method of claim 16, wherein the transformed cell comprises a therapeutic compound.

20. The method of claim 16, wherein the transformed cell is administered to the host central nervous system.

21. A method for modulating a host immune response, comprising:

administering to the host a transformed cell containing a recombinant polynucleotide comprising a promoter operably linked with a polynucleotide coding for an immunostimulatory cell surface polypeptide,

wherein the administration stimulates a host immune response to the transformed cell.

22. The method of claim 21, wherein the cell expresses, on the cell surface, a second antigen, such that the host produces an immune response against the second antigen.

23. The method of claim 22, wherein the cell expresses the second antigen from a recombinant polynucleotide.

24. A method for ablating target cells from a host, comprising:

(a) introducing into the target cell a recombinant polynucleotide comprising a promoter operably linked with a polynucleotide coding for an immunostimulatory cell surface polypeptide, wherein the immunostimulatory cell surface polypeptide

(i) activates phagocytes; but

(ii) does not fix complement;

(b) expressing the immunostimulatory cell surface polypeptide in the target cell, wherein the target cell is in the host;

wherein the expression of the immunostimulatory cell surface polypeptide by the target cell in a host induces a host phagocyte-mediated ablation of the target cell.

25. The method of claim 24, wherein the cells are tumor cells.

26. The method of claim 24, wherein the expression of the immunostimulatory cell surface polypeptide is constitutive.

27. The method of claim 24, wherein the expression of the immunostimulatory cell surface polypeptide is induced.

28. A method for treating an autoimmune disorder in a host, comprising:

(a) administering, to a host with an autoimmune disorder, transformed cells containing a recombinant polynucleotide comprising a promoter operably linked with a polynucleotide coding for an immunostimulatory cell surface polypeptide;

(b) expressing a therapeutically effective amount of immunostimulatory cell surface polypeptide from the recombinant polypeptide;

(c) contacting phagocytes with the immunostimulatory cell surface polypeptide, wherein the contacted phagocytes modulate the autoreactive T-cells to reduce T-cell autoreactivity in the host.

29. A composition comprising:

(a) a core comprising a transformed cell containing a recombinant polynucleotide comprising a promoter operably linked with a polynucleotide coding for an immunostimulatory cell surface polypeptide that is capable of stimulating an immune response against the cell in a host; and

(b) a jacket surrounding the core, the jacket comprising a permselective membrane.

30. A method for delivering a biologically active molecule to a patient, comprising:

implanting the patient with a capsule, the capsule having:

(a) a core comprising a transformed cell containing a recombinant polynucleotide comprising a promoter operably linked with a polynucleotide coding for an immunostimulatory cell surface polypeptide that is capable of stimulating an immune response against the cell in a host; and

(b) a jacket surrounding the core, the jacket comprising a permselective membrane,

wherein the transformed cell secretes a biologically active molecule from the capsule.

Statement Under Article 19(1)

The invention provides for the expression immunostimulatory cell surface polypeptides that are naturally type I cell surface molecules (*i.e.*, with the C-terminus projecting toward the cytosol and the N-terminus projecting away from the cell surface) as type II molecules (*i.e.*, with the N-terminus projecting toward the cytosol and the C-terminus projecting away from the cell surface) (*see* specification, pg. 2, lines 25-29). Accordingly, claim 1 has been amended to recite that a fusion polypeptide is expressed. Yu *et al.*, 6 International Immunology 791-7 (1998) ("*Yu*") does not disclose expression of a fusion polypeptide. Rather, *Yu* discloses the expression of B7 extracellular region (*see, Yu,* pg. 793, Fig. 1). Gajewski *et al.*, 156(8) J. Immunology 2909-17 (1996) discloses ("*Gajewski*") also does not disclose expression of a fusion polypeptide. Rather, *Gajewski* discloses expression of B7 from an expression vector (*see, Gajewski,* pg 2910, col. 1, para. 4).

ENT COOPERATION TREATY

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
17810-043		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 99/ 24630	21/10/1999	26/10/1998
Applicant		
CYTOTHERAPEUTICS, INC. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [X] contained in the international application in written form.

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   [ ] furnished subsequently to this Authority in written form.

   [X] furnished subsequently to this Authority in computer readble form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/24630

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 20-32, 34
because they relate to subject matter not required to be searched by this Authority, namely:
Rule 39.1 (iv) PCT - Method for treatment of the human or animal body by therapy

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
PCT 99/24630

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/13 C12N5/10 C12N15/62 G01N33/53 A61K45/00
A61K9/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YU X ET AL: "The role of B7-CD28 co-stimulation in tumor rejection." INTERNATIONAL IMMUNOLOGY, (1998 JUN) 10 (6) 791-7. , XP000891283 abstract figures 3,4 page 796, right-hand column, paragraph 2 --- -/--	1-4

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
20 March 2000	06/04/2000

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

International Application No
PC[redacted]S 99/24630

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GAJEWSKI THOMAS F ET AL: "Tumor rejection requires a CTLA4 ligand provided by the host or expressed on the tumor: Superiority of B7-1 over B7-2 for active tumor immunization." JOURNAL OF IMMUNOLOGY 1996, vol. 156, no. 8, 1996, pages 2909-2917, XP002133519 ISSN: 0022-1767 page 2909, left-hand column, line 12 -right-hand column, line 17 page 2913, left-hand column, paragraph 3 -right-hand column, paragraph 3 table 1	1-4
A	WO 96 02646 A (CYTOTHERAPEUTICS INC) 1 February 1996 (1996-02-01) page 6, line 18 -page 7, line 22 page 15, line 13 -page 24, line 6 claims 1,2,31	1-34
P,X	STABILA P F ET AL: "Cell surface expression of a human IgG Fc chimera activates macrophages through Fc receptors." NATURE BIOTECHNOLOGY, (1998 DEC) 16 (13) 1357-60. , XP002133520 the whole document	1-34

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 99/24630

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9602646 A	01-02-1996	US 5935849 A	10-08-1999
		US 5843431 A	01-12-1998
		AU 698624 B	05-11-1998
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		CN 1152938 A	25-06-1997
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